EFFECT OF ENVIRONMENTAL STRESS ON TRITICALE

DISSERTATION SUBMITTED FOR THE DEGREE OF

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IN

BOTANY

OZAIR AZIZ

DEPARTMENT OF BOTANY
ALIGARH MUSLIM UNIVERSITY
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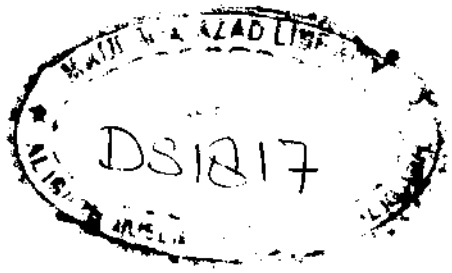

Arif Inam
M.Sc. Ph.D.(Alig)

Reader

Telephone :25676
BOTANY DEPARTMENT
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

Dated..September 1,1990...

CERTIFICATE

This is to certify that **Mr. Ozair Aziz** has worked under my supervision for the M.Phil. degree in Botany. As he has fulfilled all conditions for supplicating for the M.Phil. degree, I approve that he may submit his dissertation entitled **"Effect of Environmental Stress on Triticale"**.

(ARIF INAM)
Supervisor of Research

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Dated: Ist September, 1990 **OZAIR AZIZ**

C O N T E N T S

INTRODUCTION

CHAPTER ONE

INTRODUCTION

Cereals are the main source of food for human beings since time immemorial and are cultivated as a staple food. The main reasons for their large scale cultivation are, stability, adaptability, short span of life period and, most important, easy cultivation.

They are the main source of carbohydrate; proteins; vitamins and, to some extent, fats (Hill, 1952). Therefore, they constitute an essential food commodity for the vast majority of people in one form or another in all the parts of the world where human beings exist.

In a country like India agriculture is the main source of national income and also the main occupation; because, still after much urbanisation, more than 70% of the population live in village. The record food production of 170 million tonnes during 1988-89, surpassing the earlier best of 152 million tonnes in 1983-84, indicates the progress made by the Indian farm scientists and the farmers. But this increase on the food front does not seem to be satisfactory because during the same period, population has risen by another 50 million requiring a growing number of mouths to be fed.

Surprisingly, a myth is being floated that the country has become self sufficient in food, even though the country

continues to depend on imports of food grains. In 1988-89 alone the country imported 20.11 lakhs tonnes of wheat from U.S.A. costing $ 243.36 million and another 6.48 lakhs tonnes of rice from Thailand and Vietnam costing $ 166.76 million, etc.

It is also over looked that about one third of the population hardly meets its food requirements properly due to poverty as they are not able to afford buying it. It is estimated that 250-300 million people in this country go to bed hungry. One cannot also overlook that our population has been growing at over 2% per annum and it may touch 1,000 millions or more by 2,000 A.D. The Science Advisory Council has already cautioned that, if food production is not raised to 300 million tonnes by the end of this century, there would be an unimaginable food crisis. In view of this, it has been directed to raise the target to 185 million tonnes for 1989-90. This seems to be too ambitious and may be difficult to achieve mainly because it would largely depend on expanding the area under cultivation and increasing productivity in terms of yield per hectare. However, there are possibilities that cultivation could be improved in the dryland farming areas and by encouraging cultivation under adverse conditions. The other strategy is to increase productivity which depends on high inputs in respect of quality of seeds, increased fertilizers, pesticides, irrigation, etc.

The great need of time is to develop such varieties which can withstand various stresses, eg. of cold, drought, diseases and poor quality of soil. By crossing wheat and rye, a Scottish scientist succeeded in developing a new cereal "Triticale". It can be regarded as the future crop because of high productivity, hardiness, disease resistance, high protein as well as lysine content. Moreover, it is adaptable to unfavourable environmental conditions such as cold, sandy and acid soils (Villegas et al., 1968; Knipfel, 1969; Hulse and Spurgeon, 1974).

In the CIMMYT report on wheat improvement 1985-86, it was suggested that triticale should be tested and cultivated in major dryland areas in the developing world, particularly North Africa and the Middle East, Central India, the dry areas of Afghanistan and parts of Sind and Baluchistan provinces in Pakistan (Anonymous, 1988).

Now, triticale is known to be well adapted to various regions and climates and performes better than wheat in disease prone areas and semi tropical high lands. At present, it is grown in more than 500,000 hectares of land all over the world, including Argentina, Australia, Canada, China, Hungary, Kenya, Mexico, South Africa, Spain and the U.S.A. Some other countries, like Brazil and India, this crop is also grown (Anonymous, 1982).

In India, TL-419 has been released to the farmers of Punjab (Gill et al., 1981; Abdalla et al., 1986).

At Aligarh (U.P., India) a lot of work has been carried out on the mineral requirements of triticale by Afridi et al. (1977); Inam (1978); Abbas (1980); Alvi (1984); Ashfaq (1986); Moinuddin (1987); Samiullah et al. (1987); Fatima (1988); Haque (1989); and Moinuddin et al. (1990). However, the present author wishes to extend the study on some environmental stress aspects other than the nutrition alone.

We all know that water is a very crucial factor to ensure good harvest. In order to achieve self sufficiency in food production farmers are being encouraged to utilise all available land and irrigate it even with sub-standard water, at least in those areas where water is scarce. In industrial areas, they are even, constrained to use waste water from various industries, including refineries. This waste water may affect plant growth on the one hand while it makes the soil unfit for cultivation on the other. Water stress is a major constraint in plant functioning and induces a series of matabolic and morphological changes (Turner, 1979; Hsiao and Bradford, 1983). It is well known that water stress profoundly affects photosynthesis (Boyer, 1976; Osmond et al., 1980). Both relative water content and leaf water potential serve as indicators of plant water status (Hsiao and Bradford, 1983). Water stress decreases

the synthesis of protein as well as carbohydrate which are the main components of the cereals (Levitt, 1980).

It was therefore decided that, to start with, the effect of one of the man made environmental stresses viz. refinery waste be studied on triticale, together with wheat as check. Four field experiments have been planned on the following lines. The refinery waste water would be supplied by the Mathura Refinery of the Indian Oil Corporation, Mathura (U.P.).

(1) The comparative performance of selected varieties of triticale and one of wheat (check) in relation to germination, growth, yield and quality and cultivated using (i) waste water of the oil refinery and (ii) good quality ground water will be studied in the field, using a uniform basal fertilizer dose (N_{120} P_{60} K_{60}).

(II) The performance of one variety of triticale selected in Experiment 1 and cultivated under waste water as well as ground water conditions will be studied so as to workout its irrigation schedule under uniform basal fertilizer dose (N_{120} P_{60} K_{60}).

(III) The experiments 3rd and 4 th will also be conducted under waste water and ground water. In experiment 3rd the effect of nutritional stress on the same variety of triticale in relation to germination, growth, yield and quality will

be studied in the field.

(IV) The effect of nutritional stress on wheat (taken as check in experiment I) in relation to germination, growth, yield and quality.

REVIEW OF LITERATURE

C O N T E N T S

CHAPTER TWO

REVIEW OF LITERATURE

2.1 Triticale - history and progress

As pointed out earlier, triticale is an artificial derivative of a cross between wheat and rye. Its history is just a century old. Wilson in 1875, published the first report of a hybrid between wheat and rye. He obtained two seeds by hand pollinating emasculated wheat florets with rye pollen. But there was a serious disadvantage because of seed sterility and a further generation could not be obtained. In 1883, Carman made another cross between wheat and rye. A single hybrid plant was open pollinated and a descendent variety RNY No.6, later was grown to some extent. However, German scientist Rimpau succeeded in producing fertile hybrids of wheat and rye in 1891. In the beginning of the twentieth Century, Strampelli crossed "Rieti" wheat with rye and back crossed the F_1 with Rieti. In 1915, natural, occuring wheat x rye hybrids were described by Leighty which were found in wheat fields or in experimental plots of wheat at the U.S. Department of Agriculture Experimental Farm. Intensive research on triticale was started at Sartov Research Station (U.S.S.R.) in 1918. The wheat-rye natural hybrid Erythrospermum 46/31, in south eastern Russia represented a major contribution to the USSR economy in later years. In 1928, Meister and Tjumjakoff

produced reciprocal wheat rye hybrids. In these crosses, seed set was comparatively higher when using the wheat parent as female (Briggle, 1969).

Arne Muntzing, at the University of Lund (Sweden), began research on triticale in 1934 and has continued this work to the present time. His contributions to the knowledge of the cytology, genetics and plant improvement of triticale are outstanding. His work did much to encourage other scientists to undertake triticale research in many parts of the world. The disadvantage of sterility had been removed through the use of colchicine by Pierre Givanden (1937) in France and embryo culture techniques perfected in 1940. The first hexaploid triticale was reported by Derzhavin (1938) from a cross durum wheat x Secale montanum. A hexaploid triticale from a durum wheat x cultivated rye, S. cereale, cross by O'Mara (1948) played an important role in the development of triticale in North America and Europe. Soon, numerous new hexaploid triticales were produced from combinations of different tetraploid wheats and diploid ryes by Nakajima, 1952, 1958, 1963; Sanchez-Monge et al. 1956, 1959; Pissarev, 1963; Kiss, 1966; Larter, 1968 and Jenkins, 1969;(Zillinsky, 1974).

Discoveries of new techniques in plant breeding opened the door for the promotion of triticale. Significant contribution was made by Kiss, a Hungarian plant breeder, who started

his research on triticale in 1949. He took T. turoidum as female parent in crosses with Hungarian rye varieties and obtained his first primary hexaploid triticale in 1951. In 1952, he also produced a primary octoploid. He started crossing octoploid and hexaploid triticale in 1954 and obtained in 1960 a secondary hexaploid that was more productive than either of the parental forms (Zillinsky, 1974).

At the University of Manitoba research on triticale was started by Borlaug in 1958. Later in 1964, he initiated research in Mexico as a cooperative project between CIMMYT and University of Manitoba. This research project on triticale was finally assisted by Rockfeller Foundation.

In 1968, a very intensive selection effort was devoted to finding plants having better fertility. A few plants with improved fertility were found in the F_4 population of a cross between two hexaploid triticales. The average percentage of seed set of two of the original lines was about 6% below that of adapted bread wheat strains, and 15% above the best original hexaploid triticale. These few plants eventually provided a major break through in triticale improvement. Among the characters associated with these selections, which were later identified as Armadillo strains (Zillinsky and Borlaug, 1971), were high fertility, improved test weight, better grain yield, insensitivity to day length, one gene for dwarfness, early

maturity and good nutritional quality. A verification that a bread wheat progenitor was involved in the origin of Armadillo was obtained in 1973 when a D chromosome was found to be substituted for one of the rye chromosome (Gregory, 1973).

The weather pattern at CIMMYT's Toluca experiment station allows winter growth habit cereals to be planted in November. Thus, crosses of winter triticale x spring triticale, winter rye x spring wheat, winter wheat x spring rye and winter wheat x spring triticale can be made in the field on a large scale. When the first test of winter triticale was conducted in Ontario, Canada, in the winter of 1974-75, ninety percent of the planting was killed by cold and most of the survivors were very poor types. But one plant in ten of the surviving triticales looked as good as spring triticale grown in Mexico. These are the parent stock for continuing improvement (Anonymous, 1976b).

In 1979, international testing data continued to confirm the high yield potential of triticale, with an upto 100 percent production advantage over wheat in areas of acidic soils and cool highland production environment (Anonymous,1980).

In India also a lot of work has already been done on various aspects of triticale and Indian scientist are still engaged in its improvement and adaptability. Mention may be

made of Anand (1972) of Ludhiana; Chauhan (1972-73) of Pantnagar; Sisosida (1972-73) of Indore; Chandrappa (1973) of Karnataka; Dhiman and Kalra (1977) of Hisar; Gill et al. (1981) of Ludhiana and Sinha (1986) of IARI, New Delhi.

At Aligarh pioneering work on the mineral nutrition requirements of existing triticale varieties has been carried out by Afridi, Samiullah, and their associates (Afridi et al., 1977, Inam, 1978; Abbas, 1980, Alvi, 1984; Ashfaq, 1986; Moinuddin, 1987; Samiullah et al. 1987; Fatima 1988; Haque, 1989).

2.2 Environmental stress

Under stress conditions, potential in the environment differs from the potential within the organism in such a way that there is a driving force for transfer of energy or matter into or out of the organism, leading to biological strain. Low water potential, for example, provides a driving force for loss of water. Levitt (1980) suggested that biological stress is any change in environmental conditions that might reduce or adversely change a plant's growth or development.

Environmental stress is of two types biotic and physi cochemical. The former belongs to the field of pathology and ecology. Among the physiochemical stresses, to which a plant may be generally exposed, only water and chemical stress will be considered here (as illustrated in Fig. 1).

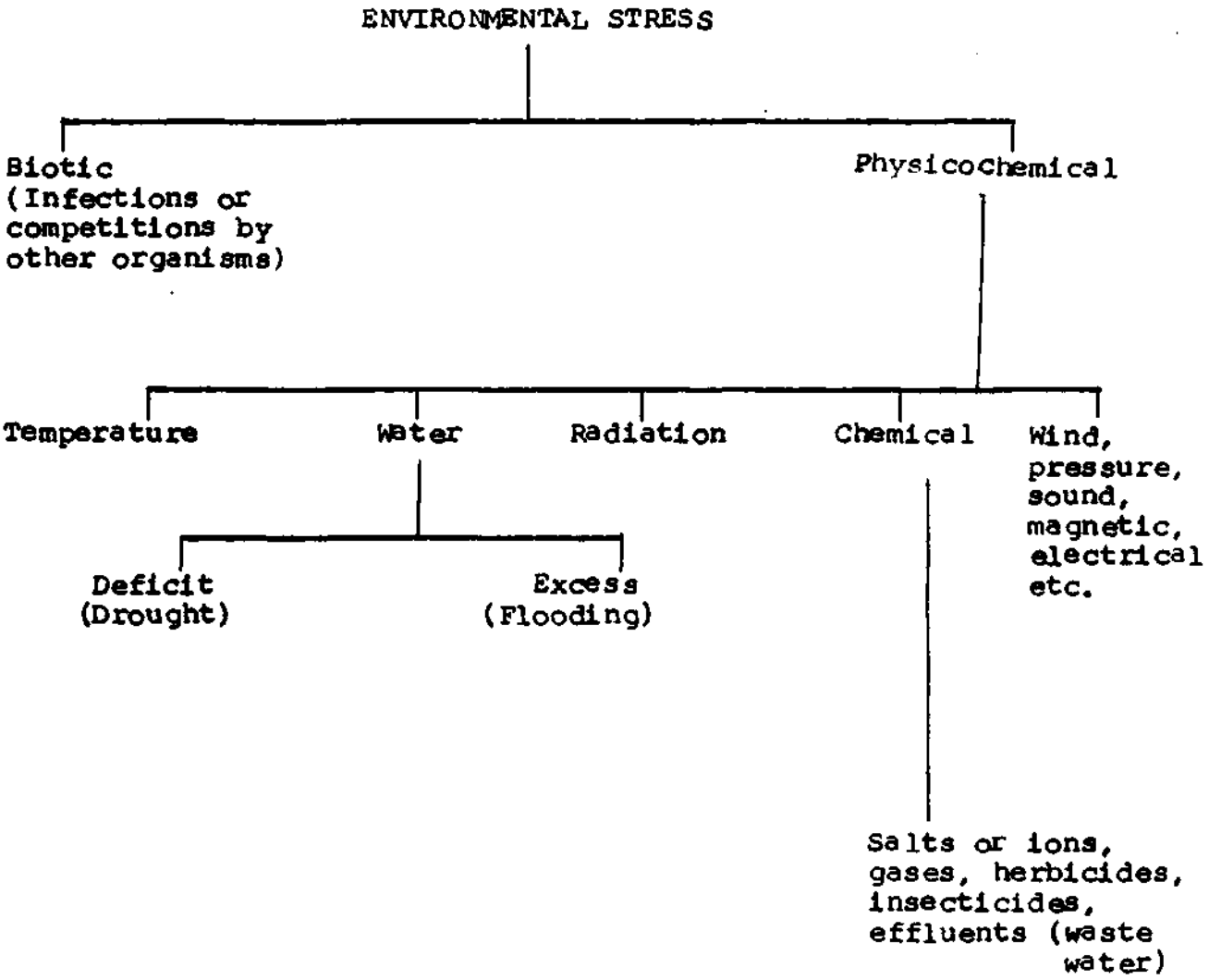

Fig. 1: Kinds of environmental stresses to which an organism may be subjected

2.2.1 Water stress: The most frequent cause of water stress in plants is a suboptimal soil moisture supply, coupled with a rate of transpiration in excess of the rate of absorption of water by roots. But, even when a plant is well watered, water stress may develop during the hot part of the day in the growing season if water absorption by roots fails to keep pace with transpiration (Noggle and Fritz, 1986).

One of the important damaging effects of water stress is the increase concentration of salts within the cells of plants subjected to water stress. These can damage the enzymes that control metabolism and are thus essential to life. An important adaptation found in many organism that are subjected to water stress is the accumulation of certain organic compounds such as sucrose and the amino acid proline that lower the osmotic potential and thus the water potential in cells without limiting enzymes function (Morgan, 1984). However, many studies indicate that activities of certain enzymes, especially nitrate reductase, decrease sharply as water stress increases. A few other enzymes, such as α-amylase and ribonuclease show increased activity. Nitrogen fixation and reduction also drop with water stress consistent with the observed drop in nitrate reductase activity (Fig. 2). Stomata also begin to close leading to a reduction in transpiration and photosynthesis (Salisbury and Ross, 1988).

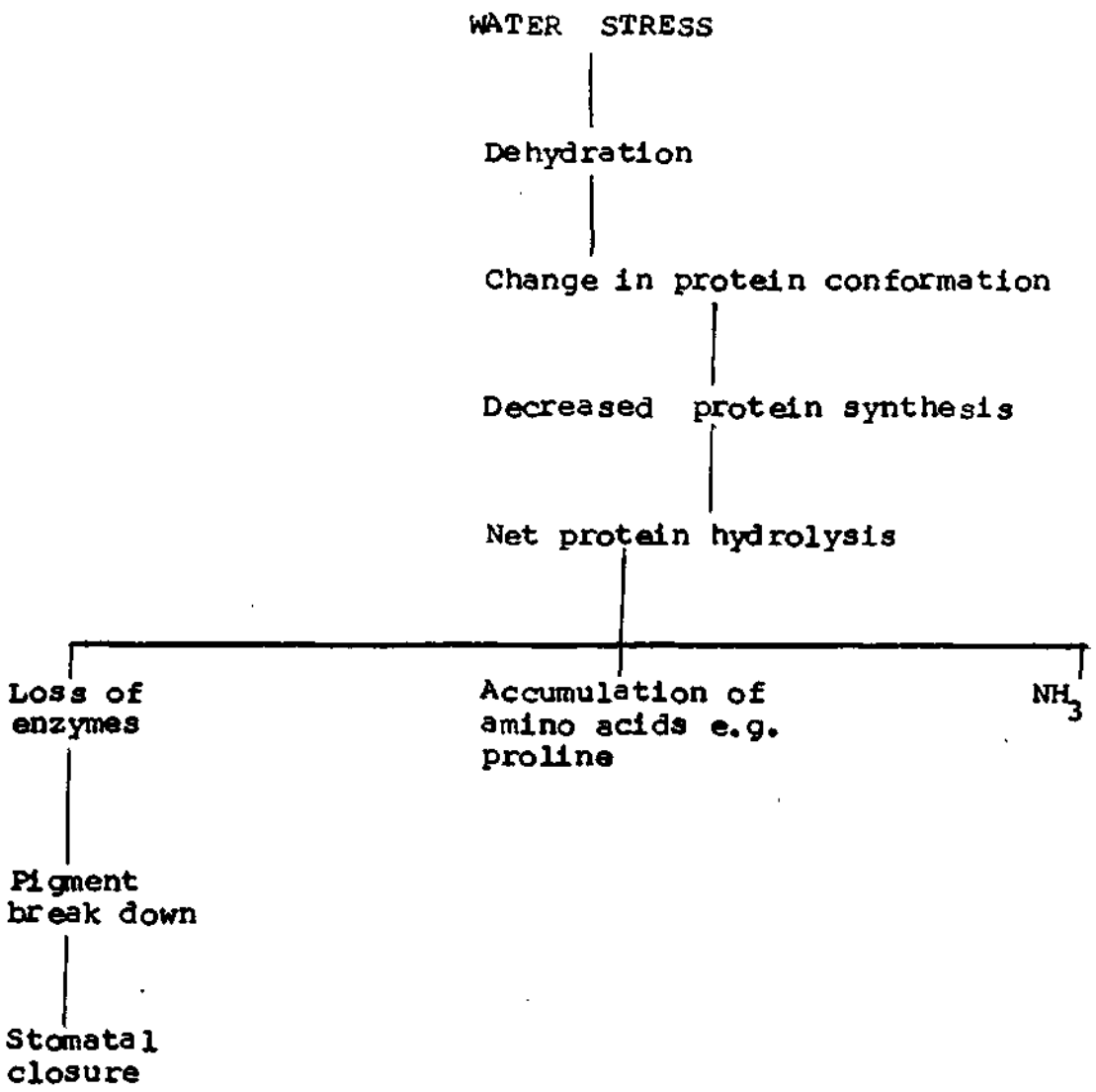

Fig.2: Kinds of effects of water stress

2.2.2 Chemical stress

Most of the toxic substances, which causes water pollution or chemical stress, are the by-products of the industries. The amounts may be relatively small but their significance is greater if they are highly toxic. The effect of a toxic substance on an organism depends on the concentration and the duration of exposure - low concentration over a long period has the same effect as a high concentration for a shorter period. Another important property of living organisms is their ability to concentrate material within their tissues. Thus, substances which occur at such a low concentration in water as to pose no threat through direct toxicity may, if absorbed, be accumulated and may go upto toxic level (Hellawell, 1988) as demonstrated in Fig. 3.

2.3 Mineral nutrition of plants

The principal function of the soil is to provide, in addition to other things, different essential inorganic nutrients. Among them, N, P and K are very important since these elements are removed by plants in very large quantities. However, other macro and micro-nutrients are equally important.

The concept of mineral nutrition dates back to the eminent Greek philosopher Aristotle (384-322 B.C.) and later

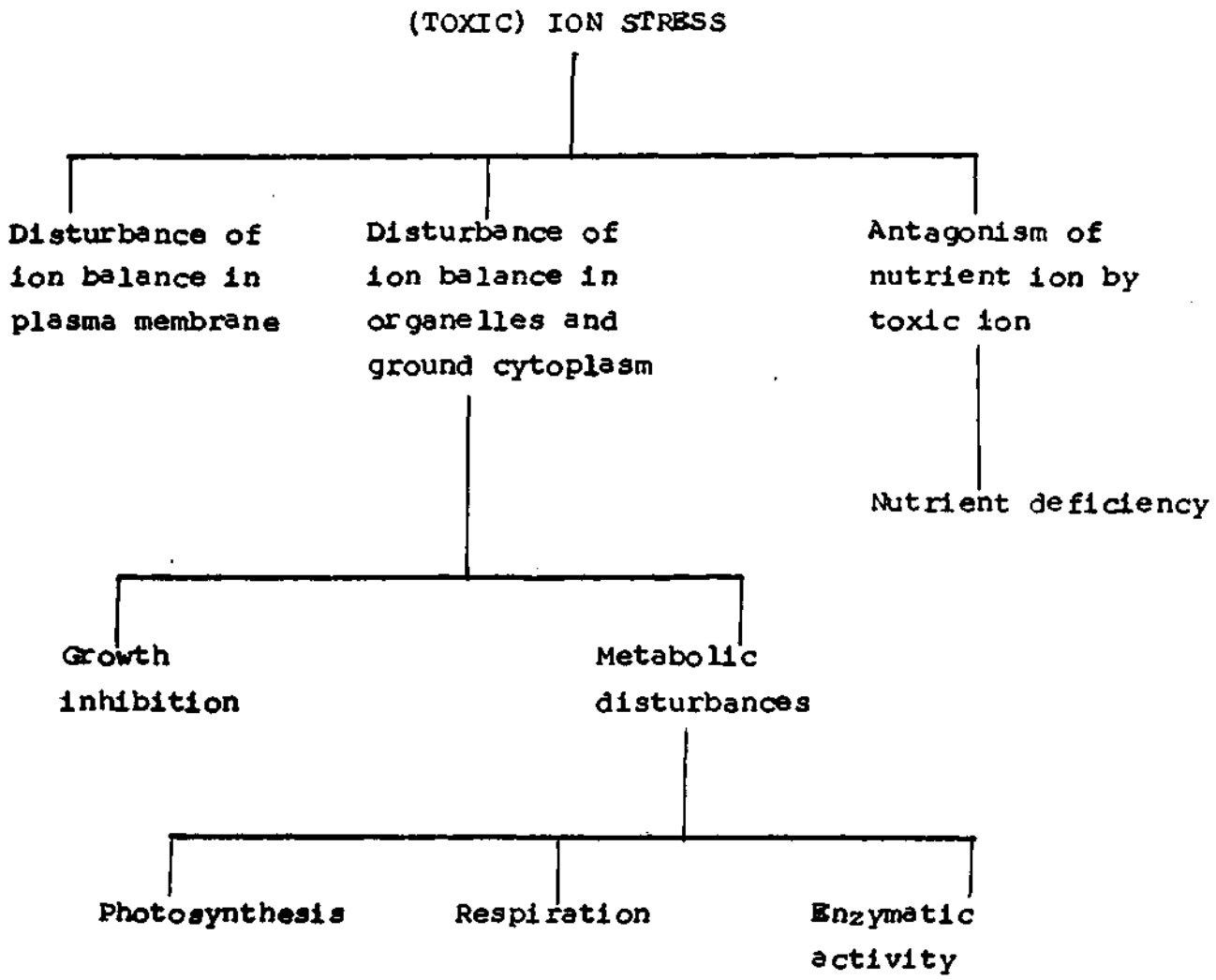

Fig.3: Kinds of ion stress effects

to the great Roman agriculturist Cato (234-149 B.C.) who emphasized the importance of ploughing and conservation of manuers (Bould, 1963).

Later, Von Helmont (1577 - 1644), Glauber (1656) and Home (1755) also added much information in the field of mineral nutrition. During the period of (1800 - 1880) that is of Liebig and de Saussure much progress was made in the study of soils and plant growth. Some other scientists, for example Boussingault in France, Lawes and Gilbert in England, Salm-Horstmar in France made significant contribution in this regard (Bould, 1963).

Later, Gregory (1937), Lundegårdh (1947, 1951), Russell (1961), Bould (1963), Hewitt (1963), Marschner (1986) wrote excellent reviews and books on mineral nutrition and opened the door for further researches in this field.

Increase in yield and grain quality of triticale by the application of N, P and K fertilizer has been reported by many workers at various locations (Kiss, 1968; Prohaszka et al., 1971; Zillinsky and Borlaug, 1971; Anand, 1972; Lafever and Schmidt, 1972; Sanchez-Monge, 1972; Acosta, 1973; Nass et al., 1975; Anonymous, 1976,a; Afridi et al., 1977; Dhiman and Kalra, 1977; Ali and Rajput, 1978; Etchevers and Moraghan, 1978; Inam, 1978; Tahir, 1978; Agarwal, 1979; Bishnoi and Mugwira, 1980; Pancholi and Bishnoi, 1981; Ponce et al., 1981; Ashfaq et al., 1983; Alvi, 1984; Ashfaq, 1986; Moinuddin, 1987 and Fatima, 1988; Moinuddin et al., 1990).

2.4 Germination as affected by water stress and waste water irrigation

Germination of the seed is the first and an important stage in the life cycle of a plant. Plant responds differently to the adverse edaphic conditions in relation to seed germination. Srivastava and Singh (1975) carried out a comparative study on wheat and triticale and found that salinity resulted in delay as well as reduction in germination.

Ashraf and Abu-Shakra (1978) in USA conducted experiment on wheat seed germination under low temperature and moisture stress. Seeds of 4 selected cultivars germinated when their moisture content was approximately 50% on a fresh weight basis. They also noted that total germination was not affected by moisture stress levels up to 12 atm., but was significantly reduced at 15 and 18 atm. osmotic tensions.

Mashhady and Sayed (1981) working in Saudi Arabia carried out experiments on wheat and triticale cultivars to salinity and irrigation intervals. They grew three wheat and one triticale in pot at different salinity levels, and noted that seed germination was not affected by salinity.

Singh (1982) working at Muzaffarnagar, studied the effect of moisture stress on two varieties of wheat and found

that seed germination and seedling growth were depressed with a decreasing external water potential in both varieties. UP 319 was more resistant to increasing water stress conditions than HD 1981.

Sayed and Mashhady (1983) in Saudi Arabia, carried out pot experiments with wheat and triticale cultivars subjected to soil salinity and soil moisture stress. They found significant differences among the varieties as far as germination was concerned.

Ajmal and Khan (1983, 1984a, 1984b, 1985) carried out very extensive work at Aligarh to study the effect of effluents from various industries, the germination of field crops, including wheat, pea, mustard, kidney bean, lady finger, pearl millet etc. They observed a general reduction in germination when the concentrations of effluents were 80-100%. They also found that germination was normal under 25-50% effluent concentration.

While working with Phaseolus radiatus, green gram, pearl millet, Betula, rice, Zea mays etc. under adverse condition in India and abroad a general decrease in germination was observed by Amakiri and Onefeghara (1984); Misra and Misra (1984); Sahai et al. (1985); Brown (1986); Kumar and Bisht (1986) and Smith et al. (1989).

2.5 Growth and yield as affected by water stress and waste water irrigation

Water is a crucial factor during all stages of the life of a plant. It acts as a medium of all reactions in the cell. Therefore , any change from normal irrigation either in the form of stress (drought, deficit) or type of irrigation water (Waste water of industries, refineries, sewage, etc.) induces a series of morphological and physiological changes in plants. More importantly, water stress reduces the growth and yield of a plant because of poor tillering, branching and stunted growth. In addition, flowering, fruiting and maturity is also delayed (Turner, 1979; Hsiao and Bradford, 1983). It also reduces the water potential of the cell (Slumelsgaard, 1976) and affects cell expansion and division (Hsiao, 1973). Sometimes, severe stress even lead to the death of plants.

According to Grifford (1979) at Canberra (Australia) when less water was available to the wheat plant, there was less response of grain yield to CO_2 enrichment.

Sionit et al. (1980) conducted experiment at Durham (U.S.A.) on the impact of repeated application of water stress on wheat. They applied water stress at 7th leaf, early anthesis and dough stages of growth and that water stress of -.25 bars at all three stress of growth reduced seed yield. The reduction

in yield was greater when the second stress cycle was also applied. Stress applied during early anthesis stage produced the smallest and least number of seeds.

Sutton and Dubbelde (1980) at Sydney (Australia) studied the effect of water stress on yield of wheat and triticale by taking two cultivars of wheat and one of triticale. Water stresses were imposed before anthesis, after anthesis and during the complete life cycle. Under continuous water deficits the cultivar of triticale produced less than the wheat (32.2 cf 53.1 g grain/bin). They also found that post-anthesis water deficits had no effect on the yield.

Gebeyehou and Knott (1983) at Addis Ababa (Ethiopia) compared the effect of water stress in the field and greenhouse during their study on durum wheat (T. turgidum) and reported decrease in grain yield, 1,000 grain weight and length of the growing period.

Sayed and Mashhady (1983) at Riyadh (Saudi Arabia) studied the performance of triticale Armadillo "S" x 308-3N and wheat Florence Aurore, Super X and Arz in saline and sodic soil under water stress. They noted that Florence Aurore was the most tolerant under moisture stress and salinity, followed by Arz and Super x on the basis of grain yield. Under sodic soils, triticale showed least range of variation.

While working on 39 varieties of triticale at the CSSRIof karnal (Haryana), Singh and Rana (1983) reported that a significant variability in grain yield and other related traits was observed in all the varieties grown in sodic soil. They also noted that the number of grains/ear and 100 grain weight showed a highly positive correlations with yield. On the other hand, the number of spikelet/ear showed a negative correlations with grain yield compared with normal fertile soil path coefficient analysis showed that plant weight, plant length and grain number/ear had direct positive effect on grain yield.

The impact of water stress on yield parameters of wheat was studied by Oosterhius and Cartwright (1983) in S. Africa. They observed that moisture stress at late vegetative stage, before the formation of spike, adversely affected the final number of fertile florets per spike. They also noted that water stress also caused death of spikelet.

Ajmal and Khan (1983, 1984a, 1984b, 1985) carried out very extensive work at Aligarh, on the effect of some industrial effluent on the soil and crop plants. In addition to some heavy metals, the effluents were rich in chloride, sulphate, sodium, potassium, calcium, magnessium etc. The values of COD and BOD were also very high. They noted that the undiluted and 75% effluent retarded the growth of plants. They also noted that

the diluted effluents (25% or less than 50%) enhanced plant growth. Biswas and Choudhury (1984) at Burdwan (W.B.) found that water stress lowered the content of chlorophyll, protein and some enzymatic activity. They further observed that stress imposed at reproductive stage reduced the grain yield comparatively more than stress at vegetative stage.

Sahai et al. (1985) studied at Gorakhpur the effect of distillery waste water on the growth behaviour of a leguminous crop (Phaseolus radiatus). The effluent was highly acidic and contained high amounts of calcium, chloride, bicarbonate, nitrogen and total dissolved solids. Its BOD value was also high. They noted that the respective lengths of the root and shoot, plant biomass, net primary productivity, seed output and chlorophyll contents were considerably increased when the plants were irrigated with 5% effluents. The soluble nitrogen and protein contents of the seed increased at effluent concentration upto 50 and 15% respectively. The distillery effluent as such was highly toxic to the growth of the plant.

Sinha et al. (1986) New Delhi, carried out experiments at I.A.R.I. on T. aestivum, T. durum and triticale under irrigated and unirrigated conditions. They noted a general reduction in the grain yield of all the cultivars. According to them, T. aestivum was more drought resistant than triticale.

On the basis of their drought susceptibility index(S) and yield potential, Aggarwal and Sinha (1987) of IARI, New Delhi, showed that varieties with low S and other moderate Y_p produced more spike/m^2 than the other irrespective of the water status. Varieties with high Y_p and low S out-yield the other varieties in irrigated and moderate drought environments because they had higher values for spike / m^2 and grain/spike. Varieties with low Y_p and high S had the lowest values for spike/m^2, grain/spike and dry matter production. The weight of grain present at the basal and middle region of the ear was also reduced when water stress was imposed in two varieties of wheat Kalyan Sona and C-306 (Bhardwaj et al. 1987). Reduction in yield & tiller number was also confirmed by Davidson (1987) in USA in wheat.

Gupta and Patil (1987) carried out extensive field work on triticale at IARI, New Delhi . During 1981-82, they observed that triticale cultivars UP7740 and UP72142 and wheat cultivar HD2204 gave 4.45, 3.99 and 3.72 t/ha average grain yield with 5, 3 and 2 irrigations, respectively at atmospheric tension 0.4, 0.6 and 0.8. During 1982-83, the average yield was 3.84, 3.65 and 3.49 t/ha, respectively. They also noted that 1,000 grain weight with 2 or 3 irrigation was higher than with 5 irrigation in 1982-83 but was not affected by irrigation regimes in 1981-82. They also, noted that increasing nitrogen rates from 0-100 kg/ha increased the yield in 1981-82 and 1982-83. However,

yields were decreased with 150 kg N/ha. Wheat cultivar HD 2 204 gave yields of 4.39 and 3.79 t/ha in the two years as compared with 3.77 and 4.90 t/ha for UP 7740 and 3.59 and 3.61 t/ha for UP 72142 triticale cultivars respectively.

Retardation in leaf area, number of tillers, ear number / m^2 and grains / ear was also observed in wheat when moisture stress imposed by Hassan et al. (1987) in Nigeria, Ivanov et al. (1987) of U.S.S.R. and Kumar et al. (1987) of H.A.W., Hisar.

Brar et al. (1988) at Ludhiana studied the genotype X environment interaction in six parental lines and all possible (15) F2 crosses of triticale. One line and four crosses showed general adaptation for yield performance while another line was specific suited to favourable environments.

Chowdhury et al. (1988) at Hisar screened sixty wheat genotypes under six different irrigation levels and found that most tolerant varieties were Narbada 4, K 7404, NP 873, LSW 131 and HS 82. Varieties N 15439, HP 1258, HS 82 and D 134, Narbada 112 and K 7404 were suitable for restricted irrigated environments.

Narang et al. (1988) of P.A.U., Ludhiana, suggested three irrigations at three growth stages (crown root initiation, jointing and boot) in place of normal four irrigations. They also reported that failure of any irrigation at any of the three

growth stages resulted in reduced grain yield being more severe at crown root initiation and boot stage particularly in late sown wheat varieties.

Talukdar et al. (1989) in Bangladesh studied yield and water use of wheat as affected by water stress. They carried out experiment in field and noted that water stress at all the growth stages reduced the grain yield and water use significantly but the effect was maximum when stress occurred from booting to flowering stages they also noted that increase in water use decreased the water use efficiency but increased harvest index.

While working on various crop plants (barley, maize, rice, sorghum and soybean) under different agroclimatic conditions in India and abroad a general decrease in growth, yield and quality, due to water stress, was also observed by Singh and Tripathi, 1972; Lewis et al. 1974; Krishnamurthy et al. 1975; Stout et al. 1978; Wilson and Allison, 1978; Cure et al. 1980; Herrero and Johnson, 1981; Kobata and Takami; 1981; Singh et al. 1981; Rahman and Yoshida, 1983; Ravindranath and Shivraj, 1983; Mahalaxmi and Bidinger, 1986; El-Zeiny and Kortam, 1987; Rego et al. 1988; Singandhupe and Rajput, 1989.

2.6 Grain quality (Protein and Lysine Content)

After going through the literature on triticale and wheat, it was noted that grain protein and lysine contents have

not been worked out in relation to water stress as well as waste water. In view of the importance of protein and lysine it was decided to review some of the important work on protein and lysine content in relation to some other aspects like mineral nutrition.

Cereals and leguminous crops are the principal source of protein. The former are the more important because they are staple food even in those areas where animal products are in plenty. Cereals contain proper amount of essential amino acids. Out of eighteen amino acids, found in natural edible proteins ten are essential for human beings. As amino acids can not be synthesized by man where lies the importance of cereals and pulses on which man has to depend.

It is not out of place to mention here that many proteins (including plant proteins) may be deficient in one or other amino acids and thus make it inferior for the human consumption. The essential amino acid in which natural protein is most seriously deficient is called the first limiting amino acid for that protein source. Lysine constitute the first limiting amino acid in most of the cereal protein. So intensive researches are going on to develop cereals with grain containing sufficient lysine. Some success came through the triticale. It contain high total protein of wheat and high lysine content of rye (Hulse and Spurgeon, 1974). Various triticale cultivar revealed a wide range of protein and lysine contents.

Villagas *et al.* (1968) CIMMYT (Mexico) analysed the protein and lysine content and reported that diploid wheat *Triticum boeticum*), contained highest protein content (19.48 to 24.80 per cent) followed by triticale (11.76 to 22.5 per cent) and rye (7.17 to 20.6 per cent). In the case of lysine content, highest content was reported in rye (2.42 to 4.26 per cent) followed by triticale 2.55 to 3.74 per cent and wheat (3.05 to 3.50).

The analysis of thirty most promising lines of triticale from CIANO (Mexico) and one hundred eight from Manitoba (Canada), were carried out at CIMMYT (Mexico). In the triticale, from CIANO, the content of protein varried from 12.77 to 17.44 per cent with average 15.20 per cent. The lysine content varried from 2.75 to 3.75 per cent with average 3.16 per cent. In Maniteba triticale grain protein ranged from 9.58 to 18.53 percent with an average of 13.98 per cent, the lysine content varried from 2.43 to 4.34 per cent with an average of 3.32 per cent(Anonymous, 1969).

Zillinsky and Borlaug (1971) also at CIMMYT (Mexico), reported that protein content of hundred triticale strains ranged from 12.0 to 21.0 per cent with a range of 0.36 to 0.72 per cent of total lysine in the grain samples. It was noted that samples containing higher proteins were low in lysine content and they concluded that inverse relationship between

protein and lysine content of protein was mainly due to the influence of environment and fertility of the soil.

Lafever and Schmidt (1972) in U.S.A. observed that the protein content of the grain varried from 14.5 per cent to 16.9 per cent in two varieties of triticale. This level of protein was higher than wheat.

Triticale line 6TA 131 was better suited to the high altitude of eastern New Mexico than wheat, barley and rye (Finkner and Fuehring, 1974). They also observed that the triticale grain contained higher protein than the other three cereals.

Hristova (1974) in Bulgaria compared the protein and lysine content of five octoploid and five hexaploid form of triticale with wheat variety Bezostaya 1 and rye variety Lozen 14. The protein in triticale was found to be 15.79 to 19.22 per cent compared with 14.90 to 12.36 per cent in wheat and rye respectively. Lysine content in triticale was 0.696 to 0.365 g per 100 g grain as compared with 0.420 and 0.371 g per 100 g grain in wheat and rye respectively.

Kies and Fox (1974) observed that out of three triticale and two wheat lines, triticale CT 4863 contained highestlysine content per 100 g of protein and highest protein percentage.

In India, Bakhshi et al. (1975) at Ludhiana, studied the protein content of thirty three triticale varieties with that of two wheat varieties namely Kalyan Sona and WL 1002. They observed that protein content in different triticale varieties ranged from 11.3 to 14.1 per cent under irrigated conditions and from 11.5 to 13.8 per cent under rainfed conditions. The protein content of six triticale strains viz TL 3, TL 10, TL 11, TL 7, TL 13 and TL 25 gave 14.1, 13.4, 13.4, 13.0, 13.0 and 13.0 per cent compared to 12.7 and 12.2 per cent given by WL 1002 and Kalyan Sona respectively in irrigated conditions. Under rainfed conditions all the varieties of triticale except TL 34 (11.5 per cent) contained higher protein content than the Kalyan Sona (11.6 per cent). It was noted that protein content of triticale strains TL 1, TL,2, TL 3, TL 4, TL 7, TL 8, TL 14, TL 26, TL 28, TL 31 and TL 33 gave significantly higher protein under rainfed conditions compared with Kalyan Sona under irrigated conditions.

Day et al. (1975) in U.S.A. carried out experiments on wheat with well water plus N_{112} P_{35} K_1 kg/ha and treated municipal waste water (no fertilizer). It was found that wheat grain that received only waste water contained more alanine, histidine, isoleucine and proline as compared to well water plus NPK.

The improved high yielding triticale strains were reported to have 10.5 to 13.5 per cent protein compared with 10.0 to 12.0 per cent in wheat grain. The lysine content in the grain protein also better ranged from 3.2 to 4.2 per cent compared with 2.3 to 3.0 per cent in bread wheat (Anonymous, 1976,C).

Dorofeev (1976) while working in Russia pointed out that protein content in triticale varried from 17 to 19 per cent compared with 14 per cent in wheat and the lysine content in triticale ranged from 3.0 to 4.0 per cent compared with 2.6 per cent in wheat.

Demir et al. (1978) observed that the triticale contained protein and total lysine content higher than wheat and rye. Lysine content was 3.6 per cent which was equal to rye.

Pleshkov et al. (1974) in Russia, Gustafson et al. (1981) in Canada and May (1981) in Australia also reported higher protein content in triticale in comparison to wheat, rye and barley.

Drossopoulos et al (1985) in Athens pointed out that glutamine, alanine, leucin, serine, valine asparagine and γ-amino butyric acid accumulated to a greater extent during water stress but was less marked in comparison with proline in two cultivars of wheat.

Very recently Moinuddin et al.(1990) studied the physico agronomic performance of triticale (Bronco 90, Badger PM 118, TL 419, Tigre 'S', Juppa 'S' Muskox 'S',Mula 'S', Delfin), and compared with wheat (HD 1982) and rye (Russian rye). They observed that cultivar Tigre 'S' and Delfin contained higher protein content than wheat and rye while TL 419, Tigre 'S' Delfin and Muskox 'S' contained higher carbohydrate content when compared to wheat and rye.

2.7 Proline Content

Proline is one of the important constituents of biological proteins which is synthesized from glutamic acid (Streeker, 1957). The accumulation of proline was observed in various crops grown under various stress conditions such as drought, salinity, S and P mineral deficiency and crown gall of tobacco and tomato diseases (Thompson et al. 1960; Seitz and Hochster, 1964; Roultey, 1966; Stewart et al. 1966; Singh et al. 1972; Stewart and Lee, 1974; Storey and Wynjones 1978).

Its degree of accumulation was suggested as parameter for drought resistance (Barnett and Naylor, 1966; Singh et al. 1972). Varieties of barley which accumulated more proline tended to survive extreme stress more readily (Singh et al. 1973 a, 1973 b).

Blum and Ebercon (1976) found that during the moisture stress there was increase in the proline content, but on rewatering there was a general decrease in proline content.

Singh and Singh (1982) at Muzaffarnagar conducted studies on two cultivars i.e. HD 1981 (drought resistant) and UP 319 (drought sensitive) of wheat. Seeds were germinated in distilled water then transferred to various stresses created by PEG resulted in considerable rise in proline accumulation in both the varieties of wheat. The relative proline accumulation increased with increasing stress levels. The accumulation of free proline was more in HD 1981 at various stress levels and periods as compared to UP 319.

Sairam and Dube (1984) at Almora found that wheat accumulated high amounts of proline when there was moisture stress and there were varietal differences in the extent of proline accumulation. Varieties that accumulated more proline under moisture stress showed symptoms of wilting at much lower soil moisture regimes than those which accumulated less proline.

Patel and Vora (1985) studied the proline accumulation in wheat, Plantago, Papaver and Mustard. Plants were grown in field with low to high water content and were subjected to water stress. They found that water stress enhances the proline content in all plants studied.

Conclusion

Considering the entire literature reviewed, the present author feels that triticale cultivars were not tested and cultivated in relation to waste water. So it is desirable to test the yielding ability and grain quality of some selected varieties of triticale after applying the refinery treated waste water.

MATERIALS AND METHODS

C O N T E N T S

CHAPTER THREE

MATERIALS AND METHODS

To fulfill the aims and objectives mentioned earlier (Chapter 1) four field experiments will be conducted taking various varieties of triticale and one variety of local wheat as check.

3.1 Preparation of experimental field

Before the start of each experiment the field will be thoroughly ploughed to ensure proper aeration. Sufficient quantity of farm yard manure will be added to maintain proper fertility and water holding capacity of the soil. Each plot will be of 10 sq.m. One light irrigation will be given before sowing of each experiment to provide the required moisture for proper germination. The basal doses of various inorganic fertilisers containing N, P and K will be added before sowing in each plot according to the aim and objective of each experiment.

3.2 Field experiments

Following four field experiments will be conducted at the Experimental Farm of Mathura Refinery, Indian Oil Corporation, Mathura (U.P.).

3.2.1 Experiment 1

This experiment will be conducted during the "rabi" season to test the comparative performance of selected varieties of hexaploid triticale and one variety of locally popular wheat. The triticale varieties will be obtained from CIMMYT Mexico, and PAU, Ludhiana. The wheat variety will be obtained from Government Agricultural Farm, Aligarh.

The design of the experiment will be split-plot with three replications (Table 1). Main-plot will consist of two types of irrigation water, i.e. ground water and treated waste water from Mathura Refinery, Mathura. Sub-plots will include varieties of triticale and wheat. Urea (120 Kg N/ha), Superphosphate (60 Kg P/ha) and muriate of potash (60 Kg K/ha) will be used as the source of nitrogen, phosphorus and potassium respectively. The seed rate will be kept uniform at 50 Kg/ha. Sowing will be done during 10-15 November by dibbling method. Weeding will be done twice at tillering and heading stages of growth of the crop. Three irrigations will be given according to the requirement of the crop.

3.2.2 Experiment 2

This experiment will be conducted in the following rabi season on the best triticale vatiety selected from Experiment. The aim of this experiment will be to work out

Table 1. Scheme of treatments (Experiment 1)

Sub-plots	Main-plots	
varieties	Treated water	Ground water
V_1	+	+
V_2	+	+
V_3	+	+
V_4	+	+

N.B. 1. Uniform basal fertilizer dose (N_{120} P_{60} K_{60}) will be given at the time of sowing.

2. V_1, V_2 and V_3 will be triticale varieties and V_4, a wheat variety.

the performance of triticale under varying levels of irrigation including no irrigation (stress). The design of the experiment will be split-plot with three replicates (Table 2). Main-plots will consist of two types of irrigation water, i.e. ground water and treated waste water from Mathura Refinery, Mathura. Sub-plots will include different levels of irrigations. Sources of NPK, date of sowing, basal doses of NPK will remain same as in Experiment 1.

3.2.3 Experiment 3

This experiment will be conducted during the following 'rabi' season. The aim of this experiment will be to study the effect of nutritional stress on triticale variety selected in Experiment 1. The design of the experiment will be split-plot with three replications /The ground water and treated water (Table 3) irrigation comprise main-plot treatment. The fertiliser treatment in the sub plot will include no fertiliser (stress), ¼ fertiliser, half fertiliser, ¾ fertiliser and full fertiliser at the time fo sowing. The sources of NPK, seed rate, sowing method and other cultural practices will be same as in earlier experiments.

3.2.4 Experiment 4

This experiment will be conducted simultaneously with third experiment. The aim of this experiment will also

Table 2. Scheme of treatments (Experiment 2)

Sub-plots	Main-plots		Remarks
Irrigation	Treated water	Ground water	
I_o	-	-	Stress
I_1	+	+	One irrigation at 30 DAS
I_2	+	+	Two irrigation at 30,60 DAS
I_3	+	+	Three irrigation at 30,60,90, DAS
I_4	+	+	Four irrigation at 30,60,90,120 DAS

N.B. Uniform basal fertiliser dose (N_{120} P_{60} K_{60}) will be given at the time of sowing.

Table 3. Scheme of treatments (Experiment 3)

Sub-plots	Main-plots		Remarks
Fertilizer doses	Treated water	Ground water	
F	+	+	Full dose of the basal fertilizer
$F_{\frac{1}{4}}$	+	+	One fourth of the full basal dose
$F_{\frac{1}{2}}$	+	+	Half full basal dose fertilizer
$F_{\frac{3}{4}}$	+	+	Three fourth of the full basal dose
F_{o}	-	-	No fertilizer (stress)

N.B. Uniform four irrigations will be given at 30,60,90 and 120 days after sowing.

be, to study the nutritional stress on wheat taken in Experiment 1. The design of the experiment will be split-plot with three replicates (Table 4). The main-plot treatments and sub-plot treatments will be the same as in Experiment 3. The sources of NPK seed rate, sowing method and other cultural practices will be same as in earlier experiments.

3.3 Sampling

The samples of soil will be collected before sowing and after harvest of each experiment, while the samples of water will be collected before each irrigation in all the experiments. On the other hand the samples of plant material will be collected at different growth stages.

3.3.1 Sampling of plant material

Random sampling of three plants from each plot will be done at various growth stages of crop developments and once at harvest to evaluate the growth, yield and quality characteristics of the crop.

3.3.1.1 Growth Characteristics

The following growth characteristics will be observed at tillering, heading and milky grain stages.

Table 4. Scheme of treatments (Experiment 4)

Sub-plots	Main-plots		Remarks
Fertilizer doses	Treated water	Ground water	
F	+	+	Full dose of basal fertilizer
$F_{\frac{1}{4}}$	+	+	One fourth of the full basal dose
$F_{\frac{1}{2}}$	+	+	Half full basal dose fertilizer
$F_{\frac{3}{4}}$	+	+	Three fourth of the full basal dose
F_o	-	-	No fertilizer (Stress)

N.B. Uniform four irrigations will be given at 30,60,90 and 120 days after sowing.

1. Shoot length / plant (cm)
2. Leaf number / plant
3. Tiller number / plant
4. Fresh weight / plant (g)
5. Dry weight / plant (g)
6. Leaf area index (LAI)
7. Net assimilation rate (NAR)

Leaf number would be a measure of differentiation and tiller number of meristematic activity, while fresh weight and dry weight would account for total productivity in terms of rate of increase of weight, volume and dry matter accumulation.

3.3.1.2 Leaf area index (LAI)

Leaf area index is the area of a plant occupying unit area will be calculated by using the following formula suggested by Watson (1947).

$$\text{Leaf area index} = \frac{\text{Leaf area per plant}}{\text{Area occupied per plant}}$$

3.3.1.3 Net assimilation rate (NAR)

The net assimilation rate is defined as the increase in weight per unit time per unit leaf area present. Net assimilation rate will be calculated according to the following formula given by Milthorpe and Moorby (1979).

$$NAR = \frac{(W_2 - W_1)}{(t_2 - t_1),} \times \frac{2.303\ (\log_{10}L_2 - \log_{10}L_1)}{L_2 - L_1}$$

W_1 = dry weight of plant at I growth stage

W_2 = dry weight of plant at II growth stage

L_1 = Leaf area of plant at I growth stage

L_2 = Leaf area of plant at II growth stage

t_1 = days of sampling at I growth stage

t_2 = days of sampling at II growth stage

Log_{10} = logarithm to base 10.

These characteristics will reflect photosynthetic efficiency, rate of differentiation and accumulation of metabolic products in plants.

3.3.1.4 Yield characteristics

Following yield characteristics will be observed at harvest. Sampling will be done by taking three plants from each plot and total grain and straw yield of each plot will be noted.

1. Ear number / plant
2. Ear weight / plant (g)
3. Ear length / plant (cm)
4. Spikelet number / ear
5. Grain number / ear
6. 1000 grain weight (g)

7. Grain yield (q/ha)
8. Straw yield (q/ha)
9. Harvest index

After harvesting, the produce will be allowed to dry for few days and the weight of the total produce (straw + grain) of each plot will be recorded. The grain in each treatment will be threshed out manually and its weight will be recorded. Straw yield will be obtained by substracting the grain yield from weight of the total produce recorded before threshing.

3.4 Chemical analysis

The plant, soil and irrigation water will be analysed for various physico-chemical characteristics.

3.4.1 Chemical analysis of Plant

The plant will be subjected to various chemical analysis at different growth stages of the crop.

3.4.1.1 Leaf analysis

Fresh leaves at tillering, heading and milky grain stages will be analysed for proline accumulation and heavy metals accumulation will be estimated in dry leaves at milky grain stage.

3.4.1.1.1 Estimation of Proline

It will be determined in the leaf according to the method used by Bates et al. (1973).

1. Approximately 200 mg to 500 mg plant material will be homogenised in 10 ml of 3% sulfosalicylic acid and the homogenate will be filtered through whatman No. 2 filter paper.

2. 2 to 5 ml filterate will be reacted with 2 ml acid ninhydrin (Appendix PI) and 2 ml of glacial acetic acid in a test tube for 1 h at 100°C (boiling water bath) and reaction will be terminated in ice box.

3. The reaction mixture will be extracted in 5 ml to 10 ml or more of toluene after mixing vigorously with a test tube stirrer for 15-20 seconds.

4. The chromophore containing toluene will be aspirated from the aquous phase. After coming to room temperature the absorbance will be read at 520 nm using toluene for blank.

5. The proline concentration will be determined from a standard prepared by different concentrations of proline. It can be calculated either by using fresh weight or dry weight.

3.4.1.1.2 Estimation of heavy metals

50 mg dried powdered leaves will be digested with nitric acid and perchloric acid. After appropriate dilution it will be analysed for heavy metals with the help of Atomic Absorption Spectrophotometer.

3.4.1.2 Grain analysis

The powdered sample of grain will be analysed for:

1. Total carbohydrate
2. Protein
3. Lysine
4. Heavy metals

3.4.1.2.1 Estimation of carbohydrate

Soluble and insoluble carbohydrate will be extracted according to the method of Yih and Clark (1965) and estimated by the method of Dubois et al.(1956).

The dry samples will be ground to fine powder and pass through a 72 mesh sieve. The powder will be stored in polythene bags and at the time of analysis powder will be dried overnight in an oven at 80°C.

50 mg powder of each sample will be transferred to a glass centrifuge tube. 5 ml of 80% ethyl alcohol after

pipetting into the test tube will be heated on water bath at 60°C for 10 min. The sample will be cooled and centrifuged at 4,000 rpm for 10 min. The supernatant will be poured into 25 ml volumetric flask with three washings and the final volume will be made up with 80% alcohol. The residue will be preserved in the same tube for the extraction of insoluble carbohydrate. 1 ml of this extract will be transferred to a test tube and evaporated to dryness on a water bath. The test tube will then be cooled and 2 ml of distilled water will be added. The extract will be used for the estimation of soluble carbohydrate.

To the residue, 5 ml of 1.5 N sulphuric acid will be added and heated on water bath at 100°C for 2 h. This digested sample will be centrifuged, after cooling it, at 4,000 rpm. The supernatant will then be collected in 25 ml volumetric flask with three washings. The final volume will be made up with distilled water. 1 ml of the extract and 1 ml of distilled water will be taken into a test tube to estimate insoluble carbohydrate.

To each test tube, containing the extract of soluble or insoluble carbohydrate, 5 ml of 5% distilled phenol will be pipetted followed by the addition of 5 ml concentrated sulphuric acid. The test tube will be shaken well, the colour will turn into yellowish orange. Now the test tube will be cooled by placing it is chilled-water. After 30 min. the solution will be transferred into colorimetric tube and optical density will

be measured at 490 nm on a "Spectronic-20" colorimeter. A blank will run simultaneously. The carbohydrate content will be calculated by comparing the optical density of the sample with a calibration curve plotted by taking known dilutions of a standard solution of chemically pure glucose.

3.4.1.2.2 Estimation of Protein

Protein will be estimated following the method of Lowry et al. (1951).

Grain powder will be kept in an oven at 80°C overnight. Then it will be cooled. 50 mg sample will be transferred to a mortar, to which 1 ml of distilled water will be added. The powder will be ground well and transferred to a centrifuge tube with repeated washings and volume will be made upto 5 ml with distilled water. The extract will be then centrifuged at 4,000 rpm for 5 minutes and the supernatant will be collected for soluble protein.

To the residue, 5 ml of 5% trichloroacetic acid will be added. The solution will be allowed to stand at room temperature for 30 minutes with thorough shakings. It will be then centrifuged at 4,000 rpm for 10 minutes and the supernatant will be discarded, 5 ml of 1N sodium hydroxide (Appendix PII) will be added to the residue, mixing well by shaking. Then residue will be

allowed to stand in a water bath at 80°C for 30 minutes. Then it will be cooled and centrifuged at 4,000 rpm, The supernatant together with three washings with 1N sodium hydroxide will be collected in 25 ml volumetric flask. The volume will be made upto the mark with 1N sodium hydroxide and then will be used for the estimation of insoluble protein.

1 ml of water extract will be transferred to a 10 ml test tube and 5 ml of reagent C (Appendix PI) will be added. The solution will be mixed well and allowed to stand for 10 min, at room temperature and 0.5 ml of reagent E (Appendix PI) will be added rapidly with immediate mixing. After 30 min. the blue colour solution will be transferred to a colorimetric tube and the per cent transmittance will be read at 660 nm on "Spectronic-20" colorimeter. A blank will run with each sample. The soluble protein content will be estimated by comparing the optical density of each sample with a calibration curve plotted by taking known dilution of a standard solution of egg-albumen.

1 ml of sodium hydroxide extract will be transferred to a 10 ml test tube and 5 ml of reagent D (Appendix PI) will be added to it. The solution will be mixed and allowed to stand for 10 min at room temperature. 0.5 ml of reagent E (Appendix PI) will he added rapidly with immediate mixing. After 30 minutes the intensity of the blue colour solution will be measured on a "Spectronic-20" colorimeter as in the case of soluble protein.

3.4.1.2.3 Estimation for lysine content of grain protein

Lysine content in the grain protein will be estimated by the colorimetric method used by Tsai et al. (1972) and modified by Villegas for cereal grains.

1. The powdered grain samples will be defatted with hexane for 6 hours in a Soxhlet apparatus. The samples will be air dried and ground further to the fine powder (80-100 mesh) in an amalgamator.

2. 100 mg of finely ground, defatted sample will be weighed in a glass vial and 5 ml of papain solution (Appendix PII) added to it. The vials will be tightly capped so as to shake the sample to wet them thoroughly with papain solution. A blank, containing papain solution will be run simultaneously.

3. The samples will be shaken for one hour and then incubated at 65°C. After taking them out from the incubator, the samples will be shaken again for another an hour to digest them fully.

4. The hydrolysed samples after being removed from the incubator will be subjected to constant shaking while being attained to adjust to room temperature. By that time the supernatant will be clear otherwise it will be centrifuged at 3000 rpm for 5 minutes.

5. From the supernatant fraction, 1 ml aliquot will be transferred into a centrifuge tube containing 0.5 ml of carbonate buffer, followed by addition of 0.5 ml of copper phosphate suspension (Appendix PII).

6. The mixture will be shaken for 5 minutes and centrifuged to precipitate the excess copper phosphate.

7. 1 ml aliquot of the supernatant will be pipetted into a 30 ml test tube, and 0.1 ml of 2 chloro-3, 5dinitropyridine solution (Appendix PIII) will be added to it and shaken well keeping the tubes well stoppered with velvet cork.

8. The reaction will be allowed to proceed for 2 hours at room temperature, shaking the test tube every 30 minutes.

9. To acidify the reactions mixture 5 ml of 1.2 N HCl (Appendix PIII) will be added to it with proper shaking.

10. Later, 5 ml of ethyl acetate will be added, the tubes stoppered are inverted 10 times to mix well. The upper phase will be extracted by a syringe adapted with polyethylene tube. This step will be repeated 3 times.

11. The aquous phase containing DNPyr lysine will be transferred to calibrated tubes and read on the "Spectronic-20" at 390 nm against a blank.

12. The lysine content of the samples will be determined by using a standard calibration curve prepared in the following manner. It will then be calculated on protein basis.

(a) The standard curve will be prepared in a range of 0 to 200 µg of lysine per ml.

(b) Stock solution of lysine (2,500 µg/ml): It will be prepared by dissolving 62.5 mg of lysine monohydrochloride in 20 ml of carbonate buffer.

(c) The stock solution will be diluted with carbonate buffer to 250, 500, 750 and 1000 µg lysine per ml.

(d) From each of these solution, one ml will be pipetted out and 4 ml of papain solution and 15 mg per ml buffer will be added to it, then the respective concentrations of lysine will become 0, 50, 100, 150 and 200 µg/ml.

(e) From each solution a 1 ml aliquot will be pipetted out into a centrifuge tube together with 0.5 ml of the solution containing amino acid mixture, followed by 0.5 ml of copper phosphate suspension. The detailed procedure presented above will be then applied from step (5) onwards and the standard curve will be plotted.

3.4.1.2.4 Estimation of heavy metals

Heavy metals in the grain will be analysed after digesting the grain powder with nitric and perchloric acid and

then will be read at Atomic Absorption Spectrophotometer.

3.4.2 Chemical analysis of soil

The soil will be subjected to various physico-chemical characteristics.

3.4.2.1 Sampling of soil

To obtain a composite sample, small amount of soil is to be collected from the desired depth (15 cm) by means of suitable tools at least from 10 to 15 well distributed spots. Now the soil will be thoroughly mixed on a polythene sheet. Only about 500 g of this composite sample will be retained for analysis. It will be kept in polythene bags with suitable de scription and identification. Soil samples will be collected before sowing and after harvest of the crop.

The following characteristics will be studied in the composite soil sample.

1. Texture
2. Organic carbon (%)
3. pH (1:2)
4. E.C. (μ mho/cm)
5. N (mg/l)
6. P (mg/l)
7. K (mg/l)

8. Ca (mg/l)

9. Mg (mg/l)

10. CEC

Since the crop will receive ground water and treated effluent waste water, it is probable that soil may accumulate some heavy metals, if present in the water. Therefore, it will be desirabl to estimate the following heavy metals in the soil.

1. Hg

2. Pb

3. Ni

4. Cd

5. Cu

6. Cr

7. Zn

3.4.2.2 Preparation of the soil sample in the Laboratory

In the laboratory the soil sample will be spread on sheet of paper to break any large lumps with wooden pestle. Then it will be passed through a 2 mm sieve. This sample will be used for various physico-chemical characteristics of the soil.

3.4.2.3 Soil texture

It will be determined by a rapid procedure by feel or rubbing the soil between the thumb and the index finger.

For this a small quantity of the dry soil will be moistened and mixed thoroughly on a glass dish to form a soft ball and then worked untill stiff and squeezed out between thumb and fore finger.

3.4.2.4 Estimation of soil pH

It will be done with the help of the pH meter. To the 10 g of the soil 20 ml of distilled water will be added and thoroughly shaked. After 30 minutes, pH of the suspension will be observed. pH meter will be calibrated with a standard buffer of known pH.

3.4.2.5 Estimation of organic carbon

It will be estimated according to the method given by Walkley and Black (1934). 2-10 g of soil will be taken in 500 ml conical flask. To this 10 ml of 1N potassium dichromate (Appendix PIV) solution and 20 ml of concentrated sulphuric acid (Appendix PIV) will be added. After shaking for about 1-2 min., it will be kept on an asbestos mat for about 30 minutes. Now 200 ml of distilled water, 10 ml of phosphoric acid (Appendix PIV) and 1 ml of diphenyl amine indicator (Appendix PIV) will be added. A deep violet colour will be appeared, which will be titrated with N/2 ferrous ammonium sulphate solution (Appendix PIV) till the colour changes to purple and finally green. Simultaneously, a blank will be run.

3.4.2.6 Estimation of electrical conductivity

20 g of soil s will be shaken intermittently with 40 ml of distilled water in 150 ml conical flask for one hour and allowed to stand. The conductivity of the supernatant liquid will be determined with the help of salt (conductivity) bridge. The apparatus will be adjusted to a known temperature (usually 25°) of the solution.

3.4.2.7 Estimation of nitrate nitrogen

This will be done according to method of Harper (1924) given in the book "Soils and water testing methods" by Ghosh et al.

20 g of soil will be shaken continuously with 50 ml distilled water for 1 hour in a 100 ml conical flask fitted with rubber stopper. A pinch of $CaSO_4$ CaO will be added and shaken. Then the contents will be filtered through a filter paper (Whatman No.1). Now 20 ml of clear aliquot will be transferred to a 50 ml porcelain dish and will be evaporated to dryness on steam bath. Then it will be cooled and 3 ml of phenol disulphonic acid (Appendix PIV) added and will be allowed to react for 10 minutes. Then 15 ml of distilled water will be added and stir with glass rod until the residue will be in solution. After cooling the contents will be washed down into 100ml volumetric

flask. To this ammonia (1:1) (Appendix PV) will be added slowly with mixing till the solution become alkaline which will be indicated by the yellow colour due to the presence of nitrate. Then another 2 ml of ammonia will be added and finally volume will be made up (100 ml) with distilled water. The intensity of yellow colour will be read in the photoelectric colorimeter using 420 mμ (blue) filter.

Preparation of Standard Curve for Nitrate

A stock solution containing 100 ppm nitrate-nitrogen (NO_3N) will be prepared by dissolving 0.7215 g of potassium nitrate and made to one litre. This will be diluted to ten times to give a 10 ppm NO_3N solution. Aliquots (2, 5, 10, 15, 20 and 25 ml) will be evaporated on water bath to dryness in small porcelain dishes. After cooling 3 ml of phenol sulphonic acid will be added and yellow colour will be read as described above. Simultaneously a blank will also run.

3.4.2.8 Estimation of available phosphorus

To 2.5 g of soil in 100 ml conical flask, a pinch of Draco G-60 or equivalent grade of activated carbon (free of phosphorus) will be added followed by 50 ml of Olsen's reagent (Appendix PV). A blank will also be run. The flasks will be shaken for 30 minutes on shaker and then contents will be filtered through Whatman No.1. In the filterate phosphorus will be estimated colorimetrically by Dickman and Bray's (1940) method.

5 ml of soil extract will be pipetted into 25 ml volumetric flask to which 5 ml of Dickman and Bray's reagent (Appendix PV) will be poured drop by drop with constant shaking till the effervescence due to CO_2 evolution will be ceased. Now neck of the flask will be washed down and the contents diluted to 22 ml. Then 1 ml of stannous chloride solution (Appendix PV) will be added and volume made up to the mark. Now the intensity of blue colour will be read at 660 nm. on "Spectronic-20."

Preparation of standard curve for phosphorus

0.439 g of potassium dihydrogen orthophosphate (KH_2PO_4) will be dissolved in about half a litre of distilled water. To this 25 ml of 7N H_2SO_4 (Appendix PV) will be added and volume will be made to 1 l with distilled water. This will give a 100 ppm stock solution of P (100 µg P per ml). From this a 2 ppm P solution will be made by 50 times dilution. For the preparation of the standard curve different concentrations of P (1, 2, 3, 4, 5 and 10 ml of 2 ppm P sol.) will be taken in 25 ml volumetric flasks. To these 5 ml of the extracting reagent (Olsen reagent) will be added and the colour will be developed by adding Dickman and Bray's reagent and stannous chloride. The colour will be read at 660 nm. The curve will be plotted taking the colorimeter reading on the vertical axis and the amount of P (in µg) in the horizontal one.

3.4.2.9 Estimation of potassium

5 g of soil will be shaken with 25 ml of neutral normal ammonium acetate (pH 7) (Appendix PVI) for 5 minutes and will be filtered immediately through a dry filter paper (Whatman No.1). Potassium concentration in the extract will be determined in the flame photometer after setting and calibrating the instrument.

Preparation of Standard Curve for Potassium

Stock solution of 1000 ppm K will be prepared by dissolving 1.908 g kcl in 1 l of distilled water.

From the stock solution of potassium chloride (Appendix PVI) aliqu ots will be diluted (in 50 or 100 ml vol. flask) with the ammonium acetate solution to give 10 to 40 ppm of K. These will be read in flame photometer after setting zero for the blank and at 100 for 40 ppm of K. The curve will be obtained by plotting the readings against the different concentration (10, 15, 20, 25, 30, 40 ppm) of K.

3.4.2.10 Preparation of extract for Ca and Mg

100 g soil will be transferred to 750 ml flask. To this 500 ml distilled water will be added and will be shaked for about one h. Now it will be filtered through Buchner funnel.

3.4.2.10.1 Estimation of calcium

It will be analysed according to method given in Analytical Agricultural Chemistry (Chopra and Kanwar, 1982).

To the 25 ml extract 2-3 crystals of carbamate and 5 ml of 16% NaOH solution, will be added. Now it will be titrated with 0.01N EDTA (Appendix PVI) using Murexide indicator powder (Appendix PVI) till colour changes from orange red to purple.

3.4.2.10.2 Estimation of magnesium

It will be also estimated according to method given by Chopra and Kanwar (1982).

To the 25 ml extract 1 ml of NaCN (2%) will be added. Now 5 ml ammonium chloride ammonium hydroxide buffer will be added. Now it will be titrated with .01N EDTA (Appendix PVI) using eriochrome black T as indicator. Colour will be changed from green to wine red.

3.4.2.11 Estimation of CEC

10 g of soil will be placed in a flask to which 10 ml of .2N Hcl (Appendix PVI) will be added. Now it will be shaked for 1/2 h and then filtered through filter paper. To make soil chloride free, it will be repeatedly washed with distil water.

Now soil will be transferred to another flask to which 10 ml saturated KCl will be added. It will be kept overnight. Then it will be titrated with .1N NaOH (Appendix PVI) using phenolphthaline as indicator.

3.4.2.12 Estimation of heavy metals in soil

Heavy metal will be analysed, after digesting the soil with nitric and perchloric acid using Atomic Absorption Spectrophotometer.

3.4.3 Chemical analysis of irrigation water

Irrigation water will be analysed for its chemical characteristics is accordance to meet the irrigation water quality criteria.

The analysis will be carried out according to "Standard Methods for Examination of water and waste water" (Anonymous, 1975). The following parameters will be studied to ascertain the quality of irrigant.

1. Total dissolved solids (TDS) (mg/l)
2. Electrical Conductivity (E.C.)
3. pH (1:2)
4. Ca^{++}
5. Mg^{++}
6. K^{+}

7. Na^{+}
8. HCO^{-}_{3}
9. CO_{3}^{--}
10. Cl^{-}
11. SO_{4}^{--}
12. Total hardness

3.4.3.1 Sampling of irrigation water

Water from the irrigation sources will be collected in 5 litre bottle and will be stored with suitable identification. But during ordinary storage there will be some change in BOD (biological oxygen demand) value. To reduce the change it will be kept at or below 4°C and analysis will be done within 24 hours.

3.4.3.2 Estimation of total dissolved solids

100 ml of water will be taken in a tared porcelain dish. It will be evaporated to dryness on a water bath. Drying will be finished in an oven at 105°C. Then it will be cooled in dessicator and weighed. The weight of the residue will represent the total solids in 100 ml of sample.

3.4.3.3 Estimation of electrical conductivity

Samples will be directly read in conductivity meter by taking the solution into the beaker. The apparatus will be

adjusted to a known temperature (usually 25°) of the solution.

3.4.3.4 Estimation of pH

pH will be determined with the help of pH-meter. The pH meter will be checked with standard buffer of known pH.

3.4.3.5 Estimation of sodium

The determination will be carried out directly with the help of flame photometer using appropriate filters and standard curves by taking known concentrations of Na.

Standard curve for sodium

5.845 g of NaCl will be dissolved in water and volume will be maintained 1 litre which will be give 100 milliequivalents per litre of Na. From this stock solution, 5, 10, 20, 30, 40 and 50 m.e. Na/l will be prepared. A curve will be drawn by plotting the flame photometer readings on the vertical axis against concentrations of Na on horizontal axis. The concentration of the Na in the unknown sample will be read from the curve.

3.4.3.6 Estimation of potassium

The determination will be carried out directly with the help of flame photometer using appropriate filters and standard curves by taking the known concentration of K.

Standard curve for the potassium

A stock solution of 1000 ppm K will be prepared in dissolving 1.908 g of KCl in 1 litre of distilled water. Dilute solutions of 2, 5, 10, 15 and 25 ppm K will be prepared from the above stock solution. Standard curve will be prepared by plotting the flame photometer readings against concentrations of K.

3.4.3.7 Estimation of carbonates and bicarbonates

50 ml of water will be taken in a clean flask. To this 5 drops of phenolphthalein indicator (Appendix PVII) will be added. The development of pink colour will indicate the presence of carbonates. This will be titrated against 0.01N sulphuric acid (Appendix PVII), till the solution will become colourless.

To the colourless solution from above titration 2-3 drops of methyl red solution (Appendix PVII) will be added. Now titration will be continued till the colour changes from yellow to rose red. This will reflect bicarbonates concentration.

3.4.3.8 Estimation of Chloride

50 ml of sample will be taken in a flask. To this 0.5 ml $K_2 Cr O_4$ indicator (Appendix PVII) will be added Now it will be titrated with 0.1N $AgNO_3$ solution (Appendix PVII).

3.4.3.9 **Estimation of sulphate**

50 ml of sample will be taken in a flask. To this 2.5 ml conditioning reagent (Appendix PVIII) and small amount of $BaCl_2$ will be added. Shaking will be done for 1 min. and then it will read at napthalometer.

Standard curve for the sulphate

Standard sulphate solution will be made by dissolving 147.9 g Na_2SO_4 in distilled water and then it will be diluted to 100 ml. From this 10, 20, 30, 40 ppm solution will be made, and turbidity will be developed by adding 2.5 ml conditioning reagent and small amount of $BaCl_2$. This will be read at napthalometer.

3.4.3.10 **Estimation of calcium**

50 ml of water sample will be taken in a conical flask. The sample will be neutralized with acid. It will be boiled for 1 min then cooled. Now 2 ml 1N NaOH solution (Appendix PVIII) will be added to maintain the pH 12-13. Now 1-2 drop ammonium purpurate indicator (Appendix PVIII) will be added. Now it will be titrated slowly with 0.01M EDTA (Appendix PVIII).

3.4.3.11 **Estimation of magnesium**

Mg will be estimated by following formula.

mg/l Mg = Total hardness (as mg $CaCO_3$/l) -

Calcium hardness (as mg/l $CaCO_3$) x 0.243

3.4.3.12 Estimation total hardness

50 ml of the sample will be taken into a conical flask and pH will be maintained 10+1. Now it will be titrated with 0.01M EDTA (Appendix PVIII) using Eriochrome Black T (Appendix PVIII) as indicator. Pink colour will be changed to blue.

3.4.3.13 Estimation of heavy metals

Each water sample will be analyzed for heavy metals using Atomic Absorption Spectrophotometer.

3.5 Statistical analysis

The data of the experiments will be analysed statistically according to Panse and Sukhatme (1985) to ascertain the significance at 5 per cent of probability.

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APPENDIX

APPENDIX

PREPARATION OF REAGENTS

1. Reagents for the estimation of proline

Acid ninhydrin: It will be prepared by warming 1.25 g nihydrin in 30 ml glacial acetic acid and 20 ml 5 M orthophosphoric acid (407 mg/l) with agitation until dissolved. It will be stored at 4°C being stable for 24 hrs.

2. Reagents for the estimation of carbohydrate

(i) 80% ethyl alcohol

(ii) 5% distilled phenol

(iii) 1.5N H_2SO_4 : 20.40 ml conc. H_2SO_4 will be dissolved in distilled water and volume will be made upto 500 ml.

3. Reagents for protein estimation

The following reagents will be prepared.

i) Reagent A: 2 per cent sodium carbonate + 0.1 N sodium hydroxide (1:1)

ii) Reagent B: 0.5 per cent coper sulphate + 1 per cent sodium tartarate (1:1)

iii) Reagent C: Alkaline copper sulphate solution: obtained by mixing 50 ml of reagent A with 1 ml of reagent B

iv) Reagent D: Carbonate copper sulphate solution: same as reagent C, except for omission of sodium hydroxide.

v) Reagent E: 1N acid Folin reagent: 100 g of sodium tungastate and 25 g of sodium molybdate will be dissolved in 700 ml of water and kept in a 1500 ml flask. Then, 50 ml of 85 per cent phosphoric acid and 100 ml of concentrated hydrochloric acid will be added. The flask will be connected with a reflux condenser and bioled gently on a heating mantle for 10 hrs. At the end of the boiling period, 150 g lithium sulphate, 50 ml water and 3 to 4 drops of liquid bromine will be added to the flask. The reflux will be removed and the solution will be boiled for 15 minutes to remove excess bromine. It will be cooled and diluted to 1,000 ml with distilled water.

The strength of this acidic solution will be estimated by titrating it with 1N solution of NaOH using phenolphthalene as an indicator. It will be then diluted to the required strength (1N).

i) 1N NaOH: 4g NaOH will be dissolved in distilled water and finally volume will be made 100 ml.

4. Reagents for lysine estimation

(i) Papain Solution: 4 mg of papain per ml of 0.03 M phosphate buffer (pH 7.4). The solution will be filtered if necessary.

(ii) 0.05 M carbonate buffer (pH 9.0)

(iii) 0.05 M borate buffer (pH 9.0)

(iv) Copper phosphate suspension: This will be prepared as follows:

Solution A: 2.8 gm of $CuCl_2 . 2H_2O$ will be dissolved in 100 ml distilled water.

Solution B: 13.6 g of $Na_3PO_4 . 12H_2O$ will be dissolved in 200 ml distilled water.

Solution A will be poured into solution B with swirling and then the mixture will be centrifuged at 3000xg for 5 minutes to collect the precipitate. The precipitate will be washed three times in 15 ml of 0.05 M borate buffer and the pellet centrifuged after each suspension. After the third washing the pellet will be suspended in 80 ml of borate buffer. This suspension will be applicable for the two weeks if necessary.

(v) 2-chloro-3, 5-dinitro pyridine solution: This solution will be prepared fresh just pfior to its use. To obtain it, 1 ml of methanol or ethanol will be added to 30 mg of 2-chloro-3, 5-dinitropyridine.

(vi) HCl solution (1.2N)

(vii) Mixture of amino acids.

Cystine	20 mg	Phenylalanine	40 mg
Methionine	20 mg	Valine	40 mg
Histidine	30 mg	Arginine	50 mg

Alanine	30 mg	Serine	50 mg
Isoleucine	30 mg	Aspartic Acid	50 mg
Threonine	30 mg	Glutamic Acid	300 mg
Tyrosine	30 mg	Leucine	80 mg
Glycine	40 mg	Proline	80 mg

100 mg of amino acid mixture will be dissolved in 10 ml of carbonate buffer.

5. Reagents for soil chemical analysis

For the estimation of organic carbon

A. 1N potassium dischromate: 49.04 g $K_2 Cr_2 O_7$ will be dissolved in distilled water and finally it will be made to one litre.

B. N/2 ferrous ammonium sulphate: 196 g of hydrated ferrous ammonium sulphate ($Fe SO_4 (NH_4)_2 SO_4 6H_2O$) will be dissolved in distilled water. To this 20 ml of conc. H_2SO_4 will be added and finally volume will be maintained to 1 litre.

C. Diphenylamine indicator: 0.5 g diphenylamine will be dissolved in a mixture of 20 ml of water and 100ml of conc. H_2SO_4.

D. Orthophosphoric acid - 85%

E. Sulphuric acid - Not less than 96%

For the estimation of nitrate nitrogen

A. Phenol disulphonic acid: This will be prepared by taking 25 g of pure phenol (C_6H_5OH, Crystal White) in a dry conical flask (500ml) to which 150 ml concentrated sulphuric acid (nitrate free) and 75 ml AR fuming sulphuric acid (nitrate free) will be mixed carefully. Now this will be kept on boiling water bath for 2 hours. After cooling it will be stored in amber coloured bottle.

B. Liquor ammonia (1:1): Ammonia having 0.88 sp. gr. will be diluted with equal volume of water.

Estimation of available Phosphorus

A. Olsen's Reagent: 42.0 g of $NaHCO_3$ will be dissolved in distilled water to give one litre of the solution. The pH will be adjusted to 8.5 with small quantities of NaOH.

B. Dickman and Brey's reagent: 15 g of ammonium molybdate will be dissolved in 300 ml of warm water (about 60°C). Then it will be cooled and filtered if necessary. To this, 400 ml of 10N HCl will be added and finally the volume will be maintained to one litre.

C. Stannous chloride solution: 10 g of crystalline stannous chloride (LR) will be dissolved in 25 ml of concentrated

HCl by warming and will be stored in amber coloured bottle. This will be 40% $SnCL_2$ stock solution. Just before use, 0.5 ml will be diluted to 66 ml with distilled water.

D. 7N H_2SO_4: 19.6 ml concentrated sulphuric acid will be added to double distilled water and the final volume will be made up to 100 ml.

Estimation of potassium

A. Ammonium acetate solution (Neutral and Normal): Solution of 2N acetic acid (glacial) and 2N ammonium hydroxide will be prepared (by titration with standard alkali and acid) and equal volumes of the two will be mixed in a large beaker. On cooling pH will be adjusted to 7.0 with acetic acid or ammonia.

B. Potassium chloride solution: 1.90 g will be dissolved in distilled water and the final volume will be made 1 litre.

Estimation of calcium

A. .01N EDTA solution: 2 g of EDTA will be dissolved in distilled water and final volume will be made 1 l.

B. Murexide indicator powder: 0.2 g of ammonium purpurate will be mixed with 40 g of powdered potassium sulphate.

Estimation of CEC

A. .2N HCl

B. .1N NaOH

6. Reagents for the analysis of water

Estimation of carbonates and bicarbonates

A. Phenolphthalein indicator: 0.25% solution will be made in 60% ethyl alcohol.

B. Standard sulphuric acid (0.01N): .272 ml sulphuric acid will be diluted in distilled water and final volume will be 100 ml.

C. Methyl red indicator: 0.5% solution will be made in 95% alcohol.

Estimation of chloride

A. Potassium chromate indicator solution: 50 g $K_2 CrO_4$ will be dissolved in distilled water. To this $AgNO_3$ solution will be added until a definite red precipitate will be formed. After overnight stand, it will be filtered and diluted to 1 litre with distilled water.

B. Standard silver nitrate titrant (0.0141N): 2.395 g $AgNO_3$ will be dissolved in distilled water and it will be diluted to 1000 ml.

Estimation of sulphate

A. Conditioning reagent: 50 ml glycerol will be mixed in a solution containing 30 ml conc. HCl + 300 ml distilled water + 100 ml 95% ethyl propyl + 75 g NaCl.

Estimation of calcium

A. Ammonium purpurate: 150 mg dry will be dissolved in 100 g ethylene glycol.

B. EDTA 0.01M: 3.723 g EDTA dihydrate also called (ethylene dinitrilo) tetra acetic acid disodium salt will be dissolved in distilled water and diluted to 1000 ml.

C. 1N NaOH solution.

Estimation of total hardness

A. 0.01M EDTA

B. Eriochrome Black T indicator: 0.5 g dry will be dissolved in 100 g 2,2', 2" nitritotriethanol.